High-throughput genotyping by DHPLC of the dihydropyrimidine dehydrogenase gene implicated in (fluoro)pyrimidine catabolism.

Abstract:
Dihydropyrimidine dehydrogenase (DPD) is the first and rate-limiting enzyme in the degradation of pyrimidines and pyrimidine base analogs including the anticancer drugs 5-fluorouracil (5-FU) and Xeloda. A decreased DPD enzyme activity has been described in cancer patients experiencing severe and life-threatening toxicity after 5-FU treatment and distinct sequence variants in the DPD gene (DPYD) have been associated with impaired enzyme function. The most prominent mutation in the DPD deficient patient group, a mutation in the splicing donor consensus sequence of intron 14, IVS14+1g>a, resulting in a truncated protein, has been observed in the Caucasian population at frequencies as high as 0.91%-0.94%. This underlines the need for a test system for DPYD mutations in patients undergoing chemotherapy with 5-FU or with Xeloda. To set up a fast and sensitive method to identify variant DPYD alleles, we analyzed 50 healthy individuals by denaturing high performance liquid chromatography (DHPLC). A primer set spanning the whole coding region and the exon-intron boundaries of DPYD was used. In addition, a cDNA-based assay was developed to rapidly identify the 165 base pair deletion in the corresponding RNA of IVS14+1g>a mutation carriers. The optimal mutation detection was elaborated for each of the PCR
fragments. DHPLC analysis detected 5 different genetic alterations occurring in the coding region of the gene, as well as 10 intronic sequence variants, respectively. In conclusion, high-throughput screening for DPYD variants by DHPLC may be a reliable tool in the investigation of the molecular basis of DPD deficiency. Furthermore, it will help to identify patients at risk for toxic side effects upon chemotherapy using 5-FU and will facilitate individual treatment of patients.